SELECTIVE PURIFICATION OF PLANT PROTEINS WHICH CO-POLYMERISE WITH MAMMALIAN MICROTUBULES

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1. Introduction

Little is known about the microtubule proteins of higher plants despite the importance of microtubules to plant morphogenesis [1]. Microtubular proteins have, however, been isolated and characterised from animal sources [2] and lower plants [3] and it is convenient to ignore the intractability of higher plant systems by assuming that the properties of plant and animal microtubules must be identical and that universal laws of microtubule assembly apply. This might well be so, but observed characteristics of higher plant cytoplasmic microtubules suggest that such assumptions might be unwarranted for such microtubules are conspicuously more cross-bridged than animal microtubules [4] and unlike most animals and lower plants they are relatively resistant to colchicine treatment [5]. An understanding of higher plant microtubules can only be obtained by examining higher plant proteins and not by extrapolating from other systems. In yeast [6,7] and *Aspergillus* [8] molecular information has been obtained on the subunit structure of microtubules despite the lack of a rich source of microtubular proteins by taking advantage of the ability of these microtubular proteins to co-polymerise with mammalian brain tubulin. In this letter we report on the isolation of putative plant tubulins by exploting their co-assembly through 2 cycles of polymerisation/depolymerisation with mammalian brain tubulin.

Abbreviations: EGTA, ethylene glycol-bis (β-amino ethyl ether) tetra-acetic acid; MES, (2-(*N*-Morpholino) ethane sulphonic acid); PMSF, phenyl methyl sulphenyl fluoride; SDS, sodium dodecyl sulphate; Tris, Tris(hydroxymethyl) aminomethane

2. Materials and methods

Carrot cells (*Daucus carota* L; cu. Champion Scarlet Horn) were grown in suspension culture [9] and incubated with 30 μCi [U-^{14}C]leucine (the Radiochemical Centre, Amersham) for 24 h. The cells were harvested by centrifugation at 500 × *g* for 5 min, washed 3 times in MSB (0.1 M MES, 1 mM EGTA, 0.5 mM $MgSO_4$, 1 mM PMSF, 1 mM β-mercaptoethanol and 1 mM GTP). The cells were ruptured in an 'X'-press (AB Biok NACA, Sweden) and then centrifuged at 100 000 × *g* at 4°C for 1 h.

Microtubule protein was prepared from fresh pig brain by 2 cycles of the polymerisation/depolymerisation procedure in [10] and was subsequently stored at 77 K.

Co-polymerisation of pig brain tubulin and radioactive carrot cell proteins was performed by incubating 1 mg radioactive carrot supernatant protein with 6 mg pig brain tubulin in 4 M glycerol/MSB in 3 ml final vol. for 1 h at 37°C. The sample was centrifuged at 100 000 × *g* for 1 h and the precipitate was re-suspended, with a homogeniser, in 2 ml MSB and maintained at 2°C for 1 h. The solution was then centrifuged at 100 000 × *g* for 30 min and the supernatant had an equal vol. 8 M glycerol/MSB added to it and was maintained at 37°C for 1 h. The solution was then re-centrifuged at 100 000 × *g* for 30 min and the 2-times re-polymerised microtubular protein pellet was re-suspended in 0.0625 M Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 1% β-mercaptoethanol and 0.005% bromophenol blue and boiled for 3 min at 100°C.

SDS–polyacrylamide gel electrophoresis was performed as in [11] on slabs of 7.5% polyacrylamide gels run at pH 9.2, since this gives optimal separation

of the α and β subunits of tubulin. Proteins were detected as in [12]; fluorography [13] was performed using EN^{3}HANCE (New England Nuclear, Boston, MA); the gels were dried and exposed to Kodak X-Omat H film.

3. Results and conclusions

Isolated mammalian brain microtubules are composed of a major structural subunit, a heterodimeric protein composed of an α and a β subunit of mol. wt ~55 000. The 2 subunits can be separated by SDS–polyacrylamide gels; the β-subunit migrating faster than the α subunit. We have been unable to polymerise microtubules from a 100 000 × g supernatant of carrot cells using the procedure in [10] but when porcine tubulin is added to the preparation there is a selective enrichment of 2 plant proteins which co-migrate with the animal tubulins on gel electrophoresis.

Figure 1A shows a autoradiogram of a 100 000 × g supernatant of carrot cell homogenate pre-labelled with [^{14}C]leucine and fig.1B, the co-polymerised product of mammalian tubulin with the same supernatant. It can be seen that two bands from the radio-

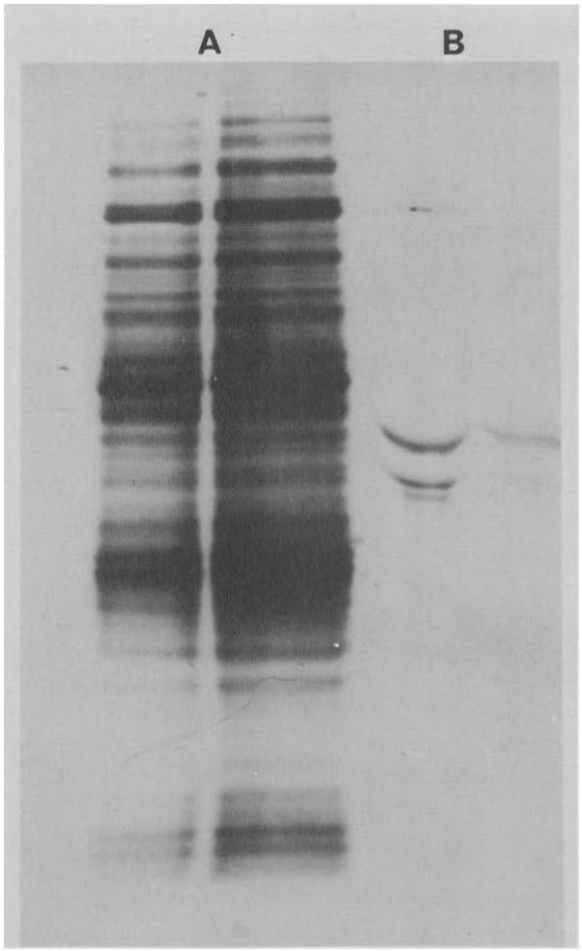

Fig.1. Autoradiogram of SDS–polyacrylamide gels. (A) 100 000 × g carrot supernatant: left lane 10.5 nCi; right lane 21 nCi. (B) The co-polymerised product of bovine brain tubulin and 100 000 × g carrot supernatant: left lane 0.87 nCi; right lane 0.43 nCi.

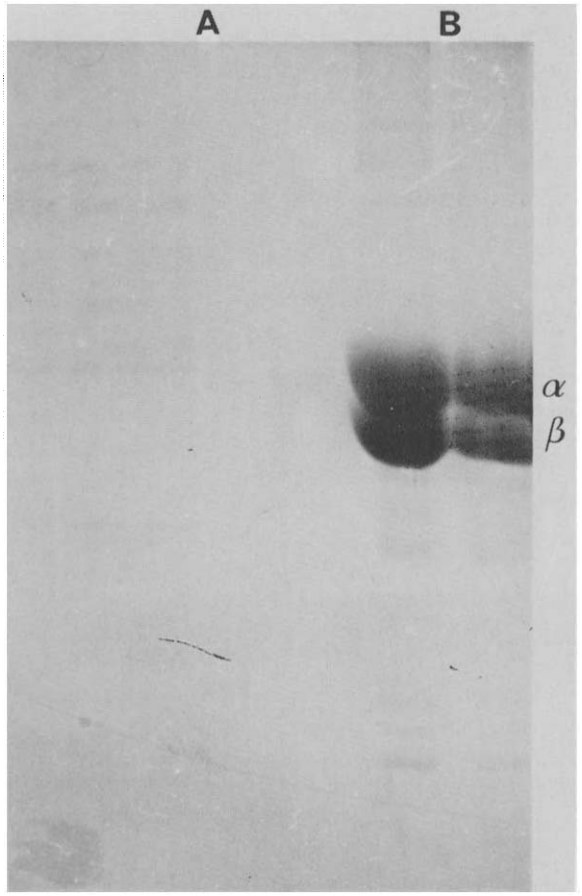

Fig.2. SDS–polyacrylamide gel of co-polymerised product of 100 000 × g carrot supernatant and animal tubulin. The sample is the same as that in fig.1 but stained with Coomassie brilliant blue to reveal proteins. (A) 100 000 × g carrot supernatant in which no protein was detected due to the low level of loading required for subsequent fluorography. (B) Co-polymerised product of mammalian brain tubulin and 100 000 × g carrot supernatant, the position of the α and β bands of tubulin are indicated.

active carrot supernatant have been selectively purified during this procedure. The presence of 2 predominant bands is consistent with their assignment as the α and β subunits of plant tubulin (fig.2).

The conserved nature of microtubular antigens amongst animal tissue has been shown [14]; this has been extended to plant tissue by demonstrating that anti-mammalian tubulin antibodies will stain plant microtubules [9,15]. Apart from antigenic similarities suggested by these observations, our findings on the co-purification of animal and plant proteins indicates that plant and animal tubulins share a common mechanism of polymerisation. In this case, differences in microtubule properties between animals and higher plants may not be due to differences in the basic subunit structure but to differences in the degree of stabilisation by associated proteins.

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